OPSONINS DISTINCT FROM OTHER ANTIBODIES.*

LUDVIG HEKTOEN.

(*From the Memorial Institute for Infectious Diseases, Chicago.*)

WHETHER the opsonic function of serum and other fluids is dependent on distinct and independent units or on antibodies with other actions as well is an interesting question concerning which there is difference of opinion.

Savtchenko[1] believed that a quantity of fixator in itself insufficient to produce solution of red corpuscles might nevertheless be sufficient to cause their phagocytosis after becoming fixed to them, and, as is well known, Metchnikoff and his followers have held that immune serum may owe its specific powers to substances that stimulate leucocytes directly to phagocytosis at the same time as bacteria or corpuscles that take up fixator are thereby made phagocytable.

In 1905[2] Dean on finding a thermostable opsonic substance in immune serum assumed on the basis of Ehrlich's theory that a small amount of such substance is present in normal serum; the diminution in opsonic power observed on heating serum, immune as well as normal, was regarded as indicating combined action by two elements, one labile and one stable. Keith[3] urged several considerations against this view and held that if amboceptor-like action is accepted, the existence of special thermostable opsoniferous groups must be admitted in view of the opsonic power of heated serum. In this case the amboceptor would combine the second and third receptor types of Ehrlich.

In a previous article on this subject[4] I concluded that the opsonins in normal and immune serum are distinct from other antibodies because a given serum may be opsonic but not lytic or agglutinating and vice versa, and because in one immune serum results had been obtained that indicated successful separation of the specific opsonic substance from the specific amboceptor.

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[1] *Ann. de l'Inst. Pasteur*, 1902, 16, p. 106.

[2] *Proc. Roy. Soc.*, 1905, 76B., p. 350.

[3] *Ibid.*, 77B., p. 536; *Aberdeen University Studies*, 1906, 21, p. 303.

[4] "Are Opsonins Distinct from Other Antibodies?" *Jour. Infect. Dis.*, 1906, 3, p. 434.

The hypothesis that hemolysis and hemophagocytosis by the same immune serum are owing to different antibodies was brought forward first by Neufeld and Töpfer.[1] In support of this hypothesis Neufeld and Bickel advanced observations to the effect that in the course of immunization hemolytic and hemotropic (opsonic) substances do not appear in the same proportions at the same time, but quite independently of each other; that the two antibodies may be separated both by heat as well as absorption in the cold; that in one case injection with alien blood gave rise to hemolytic and not to hemotropic antibody.

Neufeld's present stand seems to be this: The opsonic effect of normal serum is the result of a slight injury to the bacterium or corpuscle caused by the action of the lytic amboceptor and complement which in this case, however, do not cause lysis; immune opsonins are distinct thermostable substances, of simple nature like agglutinins; the opsonic action of heated normal serum is acknowledged but its cause is regarded as unexplained. The question that at once arises, namely, if amboceptor and complement may cause phagocytosis in the case of normal serum why not also in the case of immune serum, is discussed elsewhere in this *Journal.*[2]

Muir and Martin[3] suggest that immune opsonin may be constituted like agglutinins (because an anticoli serum containing immune body in considerable quantity had no opsonic power) but they are not willing to say that an immune body does not act as opsonin for the reason that they are not familiar with any antiserum with opsonic effect and without immune body. As pointed out in my earlier article on the identity of opsonins, antiserum for streptococci may fulfil this requirement.

Dean's[4] view is that the opsonizing action of serum is due to two substances, "the one thermostable, the substance sensibilisatrice or amboceptor; the other thermolabile, the alexin or complement. The thermostable substance is the essential one, and it may act alone, but its activity is increased by the presence of free complement. The

[1] *Centralbl. f. Bakt.*, Orig., 1905, 38, p. 456. See also Neufeld and Bickel, *Arb. a. d. kais. Gesundh.*, 1907, 27, p. 310.

[2] P. 66.

[3] *Proc. Roy. Soc.*, 1907, 79B., p. 187.

[4] *Brit. Med. Jour.*, 1907, 2, p. 1409.

amboceptor is present in relatively small quantities in normal serum, hence the apparent thermolability of the opsonin in normal serum, whereas in the case of an immune serum the amboceptor is present in a large amount, and perhaps with heightened specific properties, and plays a predominant part, and though heating results in a loss of activitity this is only partial. In both cases the loss is due to destruction of complement." Dean sees difficulty in differentiating between opsonic and lytic substances because the same substance acting in different concentrations may be capable of producing in one case opsonification and in another lysis. In another place[1] he states that a normal amboceptor may be complemented for lytic as well as opsonic action by the same fresh serum.

Wassermann[2] also announces the view that opsonin and bacterial amboceptor are identical and that it depends upon the solubility of the bacteria and on biological relations whether bacteriolysis or phagocytosis shall predominate in a given case.

Certain statements to the effect that normal opsonins are complements, based upon apparent removal of all opsonin by treatment with diverse substances that also remove or neutralize complement, cannot be accepted because of the likelihood that in many such cases the thermolabile activating element only is removed.

In the course of experiments especially in relation to hemolysis and hemophagocytosis I have found that lytic and opsonic properties quite often go together in normal serum, but normal serum may be hemopsonic without being lytic and very often smaller quantities of serum are opsonic than necessary to cause lysis. Of the various sera examined not one that is lytic for the corpuscles in question has failed to reveal some opsonic power at least when using dog leucocytes as phagocytes. The only exception to this statement is frog serum which is markedly lytic but not opsonic for rabbit corpuscles with respect to dog leucocytes. In this case, however, the lysin has the constitution of a typical toxin.[3]

I have also found various examples of persistence of opsonic power of serum after removal or destruction of the lytic amboceptor. The lytic amboceptor may be removed from serum by absorption in the

[1] *Proc. Roy. Soc.*, 1907, 79B., p. 350. [2] *Deut. med. Wchnschr.*, 1907, 33, p. 1936.

[3] Friedberger, *Centralbl. f. Bakt.*, Orig., 1907, 44, p. 32.

cold and yet the serum may be found to retain opsonic power if carefully tested. An example follows:

Antigoat rabbit serum (heated) 0.025 c.c. + normal guinea-pig serum 0.05 c.c. + 5 per cent goat corp. 1.0 c.c. = Complete Lysis.

Antigoat rabbit serum (heated and exhausted) 0.025 per cent + normal guinea-pig serum 0.05 c.c. + 5 per cent goat corp. 1.0 c.c. No Lysis.

Normal guinea pig serum 0.05 c.c. + 5 per cent goat corp. 1.0 c.c. No Lysis.

The heated and exhausted serum caused, however, marked phagocytosis of goat corpuscles by dog leucocytes.

Again, heating antihuman rabbit serum to 80° C. for 30 min. destroyed all the demonstrable amboceptor for human corpuscles but the opsonin persisted; when 0.025 c.c. of the heated serum was added to 0.2 c.c. 5 per cent suspension of human corpuscles and 0.2 c.c. of suspension of dog leucocytes, well washed, 25 per cent of the leucocytes were found to contain red corpuscles after one hour at 37° C.

Then too a serum may opsonify a certain corpuscle for one kind of leucocyte and not for some other kind. The serum of a rabbit immunized with human blood rendered human corpuscles readily phagocytable by dog leucocytes, but human leucocytes exercised no phagocytic activity under the same conditions. Human serum does not seem to sensitize for dog leucocytes for which it is markedly toxic.[1] Another example of selective action is furnished by normal rabbit serum which frequently contains an isohemopsonin that renders normal rabbit corpuscles subject to phagocytosis especially by dog leucocytes, rabbit, human, and guinea-pig leucocytes being practically inactive as a rule (Table 1).

TABLE 1.

RABBIT SERUM SENSITIZES RABBIT CORPUSCLES FOR PHAGOCYTOSIS BY DOG LEUCOCYTES BUT NOT BY RABBIT, GUINEA-PIG, OR HUMAN LEUCOCYTES.

	Leucocytes			
Serum	Rabbit	Guinea-pig	Dog	Human
0.1	Trace	0	++	0
0.05	0	0	++	0
0.025	0	0	++	0

Serum (+NaCl when necessary), 5 per cent suspension rabbit corpuscles (washed), suspension of washed leucocytes—equal parts; smears made after 90 min. at 37° C.

On testing the opsonic power of the sera of five rabbits with respect to the corpuscles of these rabbits (serum, 5 per cent suspension of

[1] Goodman, *Jour. Infect. Dis.*, 1908, 5, p. 173

corpuscles, suspension of washed leucocytes [dog] equal parts) the following results were obtained (Table 2):

TABLE 2.

ISOHEMOPSONIN (AND AUTOHEMOPSONIN) IN SERUM OF RABBITS.

	Corpuscles				
Sera	1	2	3	4	5
1	13	7	11	8	10
2	5	6	4	6	5
3	35	50	35	30	40
4	5	6	5	7	9
5	9	7	6	8	5

The figures give the percentage of phagocytic cells.

As the table indicates, normal rabbit serum may contain autohemopsonin but in a restricted sense in so far as the corpuscles are not rendered as phagocytable for the homologous leucocytes as for dog leucocytes. Each of these five rabbits were now injected intravenously with 2 c.c. of a 10 per cent suspension of washed rabbit corpuscles made up by equal amounts from the blood of the other four rabbits in the set. When the serum of these rabbits was examined nine days later the hemopsonin seemed to be markedly increased in the sera of the injected animals, but I have not been able to obtain any evidence of increase in other animals similarly injected and repeated subcutaneous injections of the animals' own blood corpuscles in increasing quantities have not produced any demonstrable opsonin for rabbit corpuscles. In no case has any isolysin been found.

In goats, however, the amount of isohemopsonin may be distinctly increased on immunization with sheep corpuscles but in no case was there any indication of isolysin in such animals.

Human serum not infrequently contains isohemopsonin (with respect to human leucocytes) but no fixed relation has been found to exist between this isohemopsonin and the isoagglutinins and isolysins.[1]

Dog serum is not destructive of anthrax bacilli because it contains only one of the elements necessary for lysis of the bacilli, namely the amboceptor. Other sera, as, for instance, rabbit serum, contain suitable complement to make the amboceptor in dog serum lytically active. Dog serum, however, is actively opsonic for anthrax bacilli, and this opsonic action is largely lost by heating the serum to 60° C. for 30 minutes but it may be almost fully restored on adding minute quantities of normal dog serum as shown in Table 3.

[1] Hektoen, *Jour. Infect. Dis.*, 1906, p. 721.

TABLE 3.

ACTIVATION OF OPSONIC SUBSTANCE FOR ANTHRAX BACILLI IN HEATED DOG SERUM.

Heated Serum (60°)	Normal Serum	Percentage of Phagocytic Cells
0.1 c.c.	.....	16
.....	0.1 c.c.	80
.....	0.005 c.c.	16
0.1 c.c.	0.005 c.c.	60
.....	NaCl only	10

All mixtures contained 0.2 c.c. of suspension of bacilli and 0.2 c.c. of suspension of washed leucocytes (dog) and sufficient NaCl solution to make the total quantity 0.6 c.c.; incubated for one hour.

At first blush these results speak in favor of the opsonins being distinct from other antibodies. At all events it is established that serum may be strongly opsonic without being lytic or containing lytic amboceptor, so far as is demonstrable with the usual methods. In the case of sera that prove to be lytic but apparently not opsonic the difficulty lies in the possibility that the proper leucocytes may not have been used. On the other side, Dean, Wassermann, and others suggest that failure to obtain lysis may be owing to the state of the object tested and not to the absence of lytic amboceptors. This consideration would apply with most effect to instances in which the bacterium or corpuscle is susceptible to lysis and to opsonification as in the case of anthrax bacilli. Here it could be said that dog complement activates the amboceptor for opsonification and that rabbit complement activates the same amboceptor for lysis. The explanation seems to fall short, however, when applied to bacteria like the streptococcus and pneumococcus that so far as we know are insusceptible of lysis yet readily opsonified. To claim that in this case also lysis is not caused by the opsonic serum on account of the physical state of the cocci would be a pure assumption.

If opsonification and lysis depend upon the same body the lytic and opsonic powers of the serum of an animal in the course of immunization should run parallel. It they do run parallel that fact would not necessarily prove that they depend upon one body, but failure to run parallel would indicate strongly dependence on more than one body. In order to study this particular point as well as to learn something of the general course of opsonin production animals were given single injections of washed alien red corpuscles and the amounts of specific agglutinin, lysin, and opsonin determined at frequent intervals for

some time afterward. The results are shown by means of curves. The figures at the left unless otherwise stated give the highest dilutions of the sera at which the special action in question was present without any doubt and the figures at the top indicate the days. All mixtures contained 0.6 c.c. and in all cases 0.2 c.c. of 5 per cent suspension of corpuscles, the rest being made up by NaCl solution plus serum in the lytic and agglutinating experiments and by NaCl solution plus serum and suspension of dog leucocytes (0.2 c.c.) in the phagocytic experiments. The usual incubation periods were used—two hours for the lytic and one hour for the agglutinating and opsonic mixtures. When the determination was made with fresh serum it was almost always made on the same day as the the serum was drawn and naturally with fresh corpuscles each time. The results of the different determinations consequently are not perhaps quite so strictly comparable as when all were made at the same time with the same corpuscular suspension, the serum from the various bleedings having been kept constantly at 0° C. In the latter case the sera were first heated to 60° C. for 30 min. and proper quantities of a suitable fresh serum used as complement for the determination of lysis. The two methods appear to yield results that are much alike. On account of certain peculiar changes to which rat corpuscles are prone when washed and suspended in salt solution, fresh suspension should be used invariably.

The charts show that in most instances the agglutinin, lysin, and opsonin curves run parallel and present the normal type of antibody curve. This is especially noteworthy in the case of the agglutinin and opsonin curves. That agglutinins and opsonins are distinct substances is indicated in several ways but most strongly perhaps by the facts that the action of agglutinins is not materially reduced, if at all, by heating serum to 60° C. for 30 minutes, and that agglutination by heated serum is not definitely promoted by small quantities of fresh serum as in the case of opsonification by heated serum. In some instances the increase above normal in the agglutinin and opsonin is disproportionately greater than that in the lysin. This is notably so in dogs after the injection of rat corpuscles. In dogs splenectomy may interfere markedly with the production of antibodies after injection of rat corpuscles but not always. In such instances

careful post-mortem examination has not revealed any accessory spleens that might have assumed whatever part the spleen may take in the production of antibodies. In the two splenectomized dogs represented on Chart 2 the lysin did not rise above normal, while the agglutinin and opsonin gave a very decided rise indeed. The indication is then, at least in this case, that the lysin is distinct from the opsonin and the agglutinin.

CHARTS

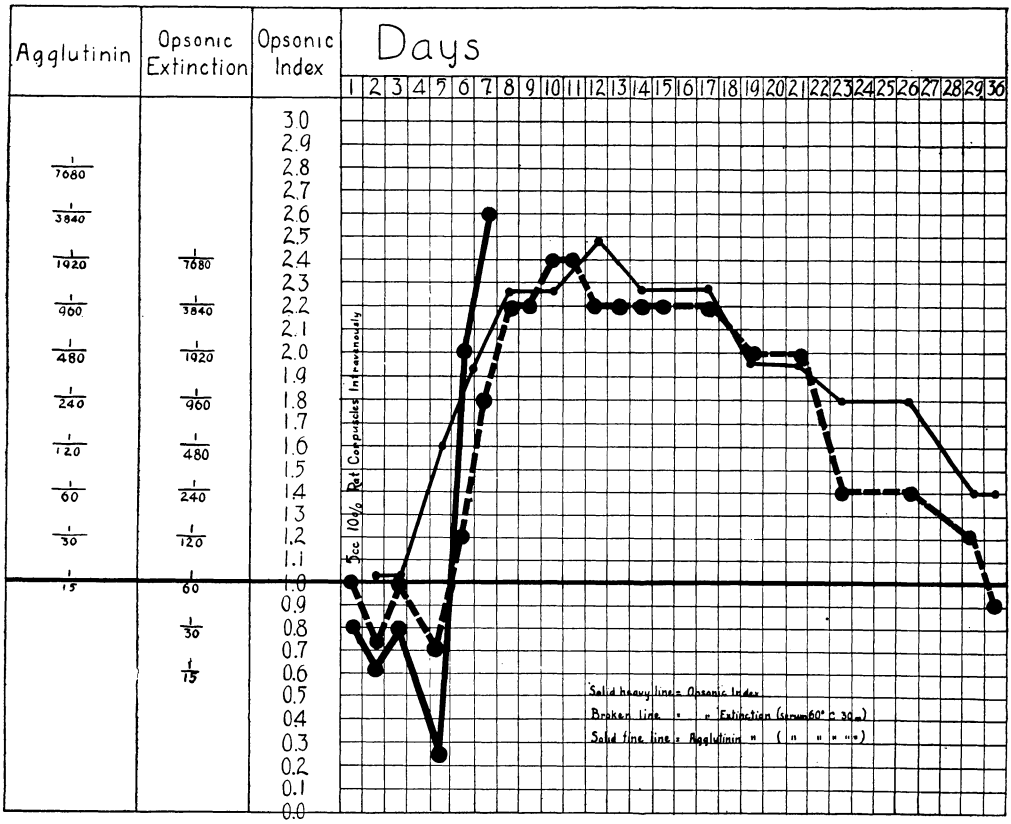
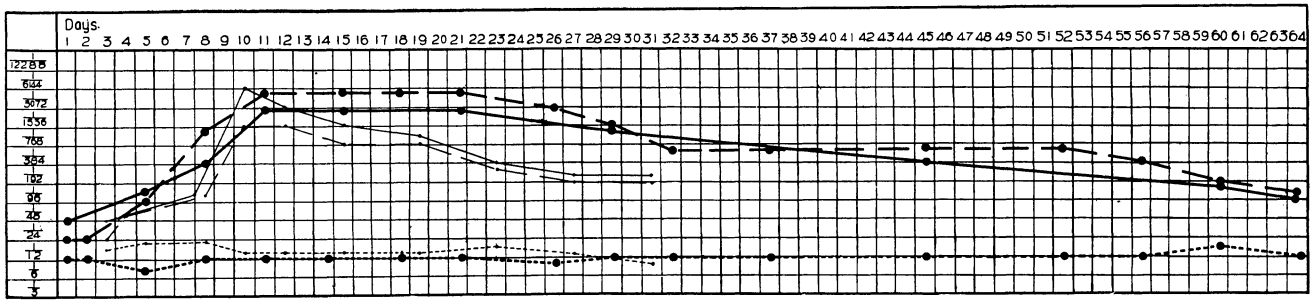
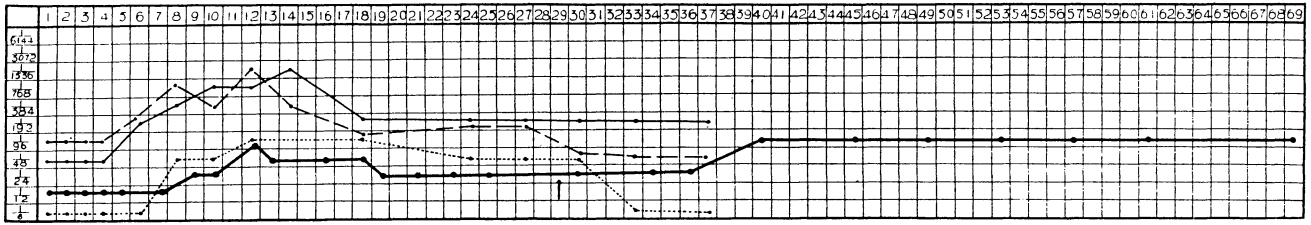


CHART 1.—Antibodies in serum of dog injected with rat corpuscles. (Opsonic index estimated by comparison of number of phagocytic mononuclear cells in mixtures with fresh serum of injected dog and in mixtures with normal dog serum under conditions of absolute comparability. Opsonic extinction of normal serum heated to 60° C. 30 m. in 60, agglutinin extinction 15.)



Heavy lines—5 c.c. 10 per cent susp. rat corpuscles intravenously and splenectomy on second day. Heated serum. Complement—.02 guinea-pig serum.
Fine lines—5 c.c. 10 per cent rat corpuscles intravenously on first day and splenectomy on fifth day. Fresh serum.
Solid lines—Opsonin; Broken lines—Agglutinin; Dotted lines—Lysin.

CHART 2.—Antibodies in serum of splenectomized dogs after injection of rat corpuscles.



Fine solid line—Opsonin; Fine broken line—Agglutinin; Dotted line—Lysin. In serum of dog splenectomized on 4th day after injection of rat corpuscles. Heated Serum. Complement. 0.013 guinea-pig serum.
Heavy line—Agglutinin and Opsonin in serum of dog injected with rat corpuscles; splenectomized on previous day. Reinjected on the 29th day.

CHART 3.—Antibodies in serum of splenectomized dogs after injection of rat corpuscles.

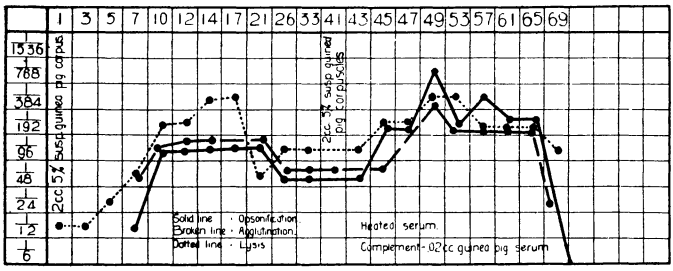


CHART 4.—Antibodies in serum of rabbit after injection of guinea-pig corpuscles.

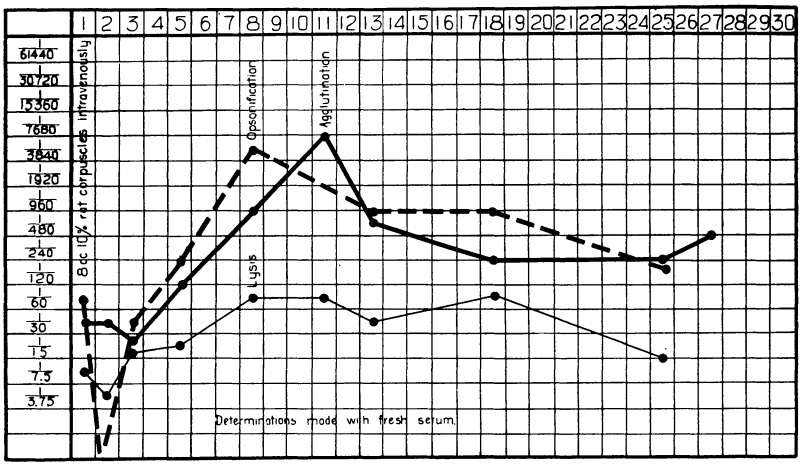


CHART 5.—Antibodies in serum of dog injected with rat corpuscles.

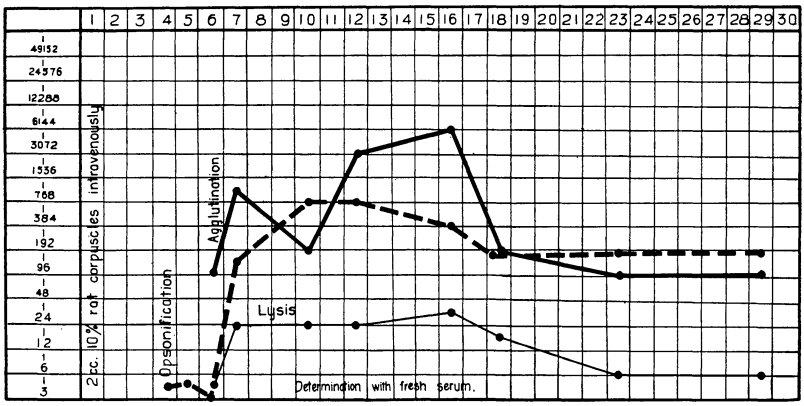
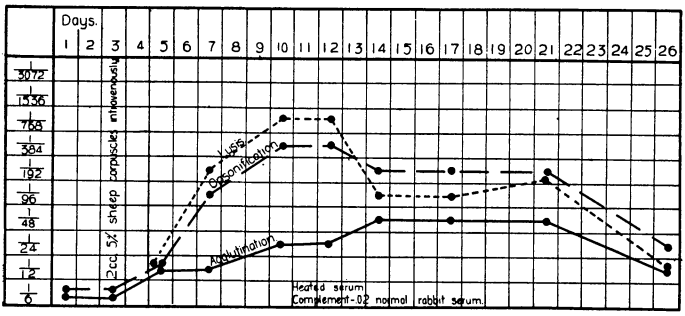
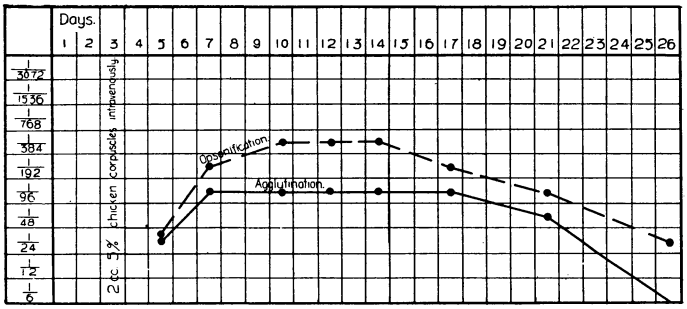
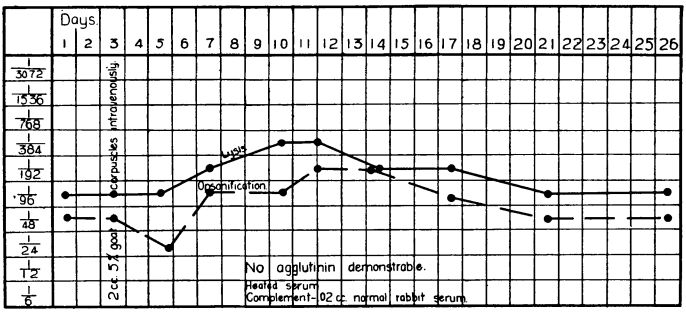


CHART 6.—Antibodies in serum of rabbit injected with rat corpuscles (no action of any kind in dilutions of 1 to 3 until fourth day).







CHARTS 7, 8, 9.—Antibodies in sera of rabbits injected with goat, sheep, and chicken corpuscles respectively.

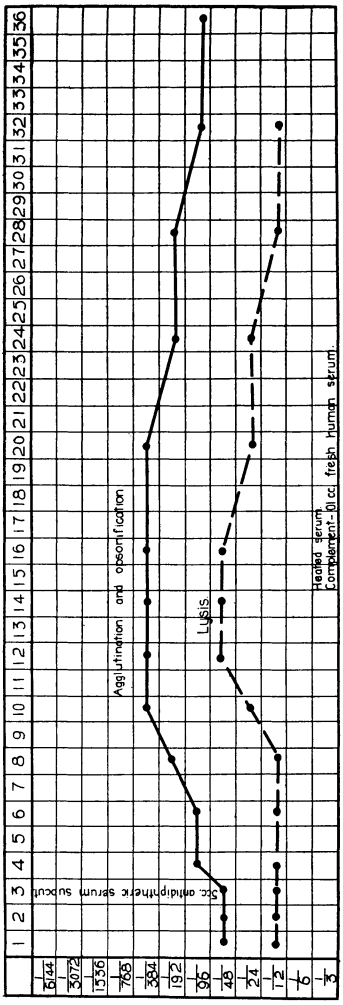


CHART 10.—Antibodies for horse corpuscles in serum of man after injection of antidiphtheric serum (mild form of serum disease, second day).